



Phylogeny, biogeography and systematic revision of plain long-nosed squirrels (genus *Dremomys*, Nannosciurinae)[☆]



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ABSTRACT

The plain long-nosed squirrels, genus *Dremomys*, are high elevation species in East and Southeast Asia. Here we present a complete molecular phylogeny for the genus based on nuclear and mitochondrial DNA sequences. Concatenated mitochondrial and nuclear gene trees were constructed to determine the tree topology, and date the tree. All speciation events within the plain-long nosed squirrels (genus *Dremomys*) were ancient (dated to the Pliocene or Miocene), and averaged older than many speciation events in the related Sunda squirrels, genus *Sundasciurus*. Within the plain long-nosed squirrels, the most recent interspecific split occurred 2.9 million years ago, older than some splits within Sunda squirrels, which dated to the Pleistocene. Our results demonstrate that the plain long-nosed squirrels are not monophyletic. The single species with a distinct distribution, the Bornean mountain ground squirrel (*Dremomys everetti*), which is endemic to the high mountains of Borneo, is nested within the Sunda squirrels with high support. This species diverged from its sister taxa in the Sunda squirrels 6.62 million years ago, and other plain long-nosed squirrels over 11 million years ago. Our analyses of morphological traits in these related genera support the re-classification of the Bornean mountain ground squirrel, *Dremomys everetti*, to the genus *Sundasciurus*, which changes its name to *Sundasciurus everetti*. Past inclusion in the plain long-nosed squirrels (*Dremomys*) reflects convergent evolution between these high elevation species.

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1. Introduction

The biota of Southeast Asia, one of the most threatened and biodiverse in the world, includes multiple biodiversity “hotspots” (de Bruyn et al., 2014; Mittermeier et al., 2005; Myers, 2003) and the world’s largest diversity of tree squirrels (Order: Rodentia; Family: Sciuridae). The subfamily Nannosciurinae alone contains 14 genera and over 60 species of tree squirrels (Thorington et al., 2012; Musser et al., 2010; Thorington and Hoffmann, 2005; Corbet and Hill, 1992). The subfamily is distributed from the Indian Subcontinent to China and across mainland Southeast Asia to the Sunda

Shelf (Malay Peninsula, Sumatra, Java, Borneo, and associated islands), and has crossed Wallace’s and Huxley’s Lines into Sulawesi and the Philippines, respectively.

Within this region, Borneo is particularly rich in both diversity and endemism. Of the eight genera of nannosciurine squirrels native to Borneo, only two are also distributed across the Isthmus of Kra into mainland Southeast Asia (Corbet and Hill, 1992; Thorington et al., 2012). One genus distributed north of the Isthmus of Kra, *Callosciurus*, is a widespread and diverse group in need of revision (Moore and Tate, 1965). The second genus, the plain long-nosed squirrels (*Dremomys*), is a genus of exclusively high elevation endemics (Thorington et al., 2012; Corbet and Hill, 1992). The plain long-nosed squirrels are drab brown animals with elongate rostra and short bushy tails, with a variable amount of red–orange fur in the pelage (Thorington et al., 2012; Corbet and Hill, 1992). Five of the six species of plain long-nosed squirrels are distributed in mainland Southeast Asia north to China and west

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to India: *Dremomys pernyi*, *D. lokriah*, *D. gularis*, *D. rufigenis*, *D. pyrrhomerus* (Corbet and Hill, 1992; Thorington et al., 2012; Thorington and Hoffmann, 2005). Some of the species have restricted ranges, and two, the red-cheeked squirrel (*D. Rufigenis*), and Perny's long-nosed squirrel (*D. pernyi*), are widespread. The last species, and the smallest member of the genus, the Bornean mountain ground squirrel (BMGS), *Dremomys everetti* (Thomas, 1890), is known only from the mountains of Borneo, far disjunct from the geographic ranges of other members of the genus (Fig. 1). A previous phylogenetic study of the species relationships in *Dremomys* did not include this species (Li et al., 2008). In the past, the BMGS has been classified in other nannosciurine genera, including *Funambulus* and *Rhinosciurus* (e.g. Allen and Coolidge, 1940; Banks, 1933). However, classification of the BMGS in *Dremomys*, based especially on its pelage and relatively long snout, has been accepted for nearly a century (e.g. Corbet and Hill, 1992; Ellerman et al., 1940; Moore and Tate, 1965; Robinson and Kloss, 1918; Thorington et al., 2012; Thorington and Hoffmann, 2005).

In the closely related genus of Sunda squirrels (*Sundasciurus*), high elevation subspecies of a widespread species (*S. tenuis*) were found to be genetically divergent from the lowland populations, and so, with morphological support, they were resurrected as independent species (*S. tahan*, *S. altitudinus*; den Tex et al., 2010; Thorington et al., 2012). These data hint at the particular taxonomic difficulty presented by high-elevation species, perhaps due

to strong abiotic selection leading to convergent phenotypes, similar to the convergence observed in American ground squirrels (Harrison et al., 2003).

Here we use nuclear and mitochondrial DNA sequences to construct a phylogeny for all recognized species of the plain long-nosed squirrels. In addition to the plain long-nosed squirrels, representatives from many closely related genera of nannosciurine squirrels were included in our phylogenetic comparisons, including 16 of the 17 species of the Sunda squirrels, and 10 species of *Callosciurus*, and a representative of *Tamiops*, *Exilisciurus* and *Funambulus*. We date this phylogeny in order to estimate when species and genera diverged, and consider the implications of the phylogeny for understanding the evolution, biogeography, and morphology of these high elevation endemic squirrels. We evaluate each genus for monophyly, and test for association of phylogenetic clades with biogeographic regions. We test the hypothesis that the high elevation environment drives convergent evolution in morphological traits, and place our results in an evolutionary context.

2. Materials and methods

2.1. Materials

Tissue samples from twenty-four squirrels were collected for this study and amplified for the three molecular markers. These

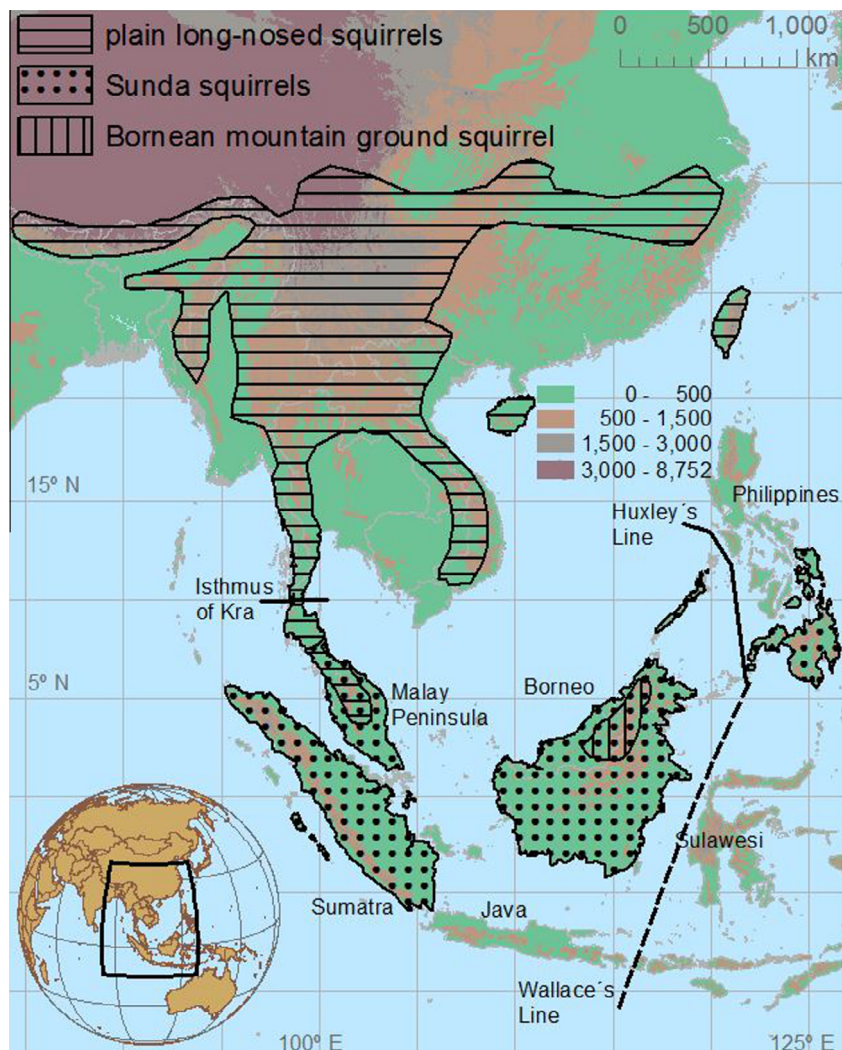


Fig. 1. Distribution of the plain long-nosed squirrels (genus *Dremomys*) and the Sunda squirrels (genus *Sundasciurus*) with the Bornean mountain ground squirrels highlighted separately. Distributions follow Thorington et al. (2012) and Corbet and Hill (1992). Relevant biogeographic breaks, islands, and elevation (in meters) are labeled.

were combined with published sequences, including 30 individuals for mitochondrial and nuclear genes, and 27 for only cytochrome *b* (Table 1). Twelve newly acquired tissue samples (nine *Dremomys everetti*, one *Sundasciurus lowii*, one *S. jentinki*, and one *Callosciurus prevostii*) from field expeditions were collected in Kinabalu Park, Sabah (Borneo), Malaysia. Specimens were collected according to the guidelines of the American Society of Mammalogists (Sikes et al., 2011), as approved by institutional animal care and use committees (Smithsonian Institution, National Museum of Natural History, Proposal Number 2012-04 and Estación Biológica de Doñana Proposal Number CGL2010-21524), with permission from Sabah Parks (TS/PTD/5/4 Jld. 47 (25)), and exported with permissions from the Sabah Biodiversity Council (Ref: TK/PP:8/8Jld.2).

An additional 12 tissue samples were obtained from museum loans from the National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (USNM); National Zoological Park, Washington, D.C., USA (NZP); Royal Ontario Museum, Toronto, Canada (ROM); and the Australian National Wildlife Collection, CSIRO, Canberra, Australia (ANWC) (one *Dremomys pernyi*, two *D. rufigenis*, one *Exilisciurus exilis*, one *Funambulus pennantii*, one *Sundasciurus tenuis*, two *S. lowii*, one *Callosciurus adamsi*, one *C. finlaysonii*, one *C. orestes*, one *C. phayrei*). Published sequences from various muroid outgroups were also included (Table 1).

2.2. Molecular work

2.2.1. DNA extraction, amplification and sequencing

DNA was extracted from tissue samples with DNeasy Blood and Tissue Kits (Qiagen) following manufacturer instructions. Whole genomic DNA concentration was standardized by spectrophotometry (NanoDrop v 2.0) to approximately 50 ng/μl.

Cytochrome *b* was amplified in two fragments with universal mammalian primers (L14724 [5'-GAT ATG AAA AAC CAT CGT TG-3'], and H15417 [5'-AGT AGG GGT GGA AAG GGA TTT-3']) and L15370 [5'-CAC GAA ACA GGA TCA AAC AAC C-3'] and H15910 [5'-GAT TTT TGG TTT ACA AGA CCG AG-3'], Kocher, 1989) which generated a 1115 base pair (bp) long fragment. PCR reactions consisted of: 1 × Gold buffer (Perkin–Elmer, ABI) 0.2 mM dNTPs, 2 mM of MgCl₂, 0.5 mM of each primer, and 1 U of Taq Gold (Perkin–Elmer, ABI) in 25 μl. Cycling conditions were: 95 °C for 10 min, two cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 1 min, two cycles of 95 °C 15 s, 58 °C for 30 s, 72 °C for 1 min, and 19 cycles of 95 °C for 15 s, 56 °C for 30 s, 72 °C for 1 min, followed by a final extension of 72 °C for 5 min.

Two nuclear markers were amplified for this study, *c-myc* and *RAG-1*. The proto-oncogene *c-myc* is a single copy gene with three exons which has been used to reconstruct mammalian, crocodylian, and avian phylogenies (e.g., Braun et al., 1985; Cole, 1986; Yaniv and Jacques, 1997) and has been demonstrated to be phylogenetically informative in squirrels (Steppan et al., 2004). For this study, we sequenced 565 bp of exon 2 of the *c-myc* gene using primers S54 [5'-GGAATATGACCTCGACTACGACTC-3'] and S55 [5'-TACAG GATTTGGCGAGCTG-3'] as in Steppan et al. (2004). The recombination activation gene (*RAG-1*) is also a single copy gene and consists of one exon uninterrupted by introns (Groth and Barrowclough, 1999). Here we analyzed a 548 bp fragment of *RAG-1* which was previously found to be phylogenetically informative for mammals, using previously published primers S70 [5'-TC CGAGTGGAAATTAAGMTGT-3'] and S105 [5'-CTCCACRGGGT CAGCCAGAAT-3'] as in Steppan et al. (2004).

All PCR products were cleaned with ExoSAP-IT (Affymetrix), then Sanger sequenced using BigDye terminator premix version 3.1 (Applied Biosystems), and separated on an ABI 3130xl automated capillary sequencer. Geneious v. 7.0.6 was used to remove primer sequences, and to edit and align sequences from all of the fragments used in the analysis.

2.2.2. Molecular phylogenetic analyses

Cytochrome *b* and nuclear gene sequence alignments were analyzed separately, as well as concatenated to reconstruct phylogenetic trees with maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods. The cytochrome *b* dataset included 82 individuals, and the *RAG-1* and *c-myc* contained 45 individuals (Table 1). The MP tree was generated using the parsimony ratchet method (Nixon, 1999) implemented in PAUPRat (Sikes and Lewis, 2001) on the concatenated dataset. The bootstrap support was calculated in PAUP v.4.0a136 (Swofford, 2003) using random taxon addition (10 replicates), tree-bisection-reconnection branch swapping, and heuristic searches with 1000 bootstrap replicates.

We used the software PartitionFinder (Lanfear et al., 2012) to determine the best partitioning scheme and model of evolution for each gene. Base composition of all ingroup taxa was tested with a chi-square (χ^2) as implemented in PAUP* v.4.0a136. The output from PartitionFinder separated cytochrome *b* into 1st, 2nd and 3rd codon positions and neither *c-myc* nor *RAG-1* were partitioned. Under both AIC and BIC, the best-fit models were: SYM + I + G (Zharkikh, 1994) for cytochrome *b* 1st codon position (which was modified into a GTR + I + G with equal base frequencies for the analyses using BEAST, see below); HKY + I + G (Hasegawa et al., 1985) for cytochrome *b* 2nd codon position; GTR + G (Tavare, 1986) for cytochrome *b* 3rd codon position; HKY + G for *c-myc*; and GTR + G for *RAG-1*. The estimated models for each gene were employed to generate a ML tree using the software GARLI (Genetic Algorithm for Rapid Likelihood Inference; Zwickl, 2006) and grid computing (Cummings and Huskamp, 2005) through The Lattice Project (Bazin et al., 2008). We used ten search replicates and branch support was assessed using 1000 bootstrap replicates.

The same model parameters were applied for the BI analysis in MrBayes version 3.2.2 (Ronquist and Huelsenbeck, 2003) on XSEDE via the Cipres Science Gateway (Miller et al., 2010). The BI analysis was run using 50,000,000 generations along four chains with two replicates at a temperature of 0.05. The convergence between the two runs was analyzed by comparing the average standard deviation of split frequencies (ASDSF) through Geneious v. 7.0.6. Sample frequency was set to 1000 with a burn-in of 50,000.

Ancestral range reconstruction was performed to estimate the biogeographic history of these squirrels. The analysis used modern distribution information (mainland Asia, Malay Peninsula, Borneo, Sumatra or the Philippines) and the concatenated dataset of cytochrome *b*, *c-myc* and *RAG-1* (and a PHyML tree as the newick input tree), for ingroup squirrels only using the software Lagrange v.20130526 (Ree and Smith, 2008). Five geographic regions were included in the analysis, A = mainland Asia, M = Malay Peninsula, B = Borneo, S = Sumatra and P = Philippines. The root age was not estimated, the adjacency matrix was left to default, a maximum of 3 biogeographic regions were allowed, and all range combinations were allowed. Dispersal constraints were left to default, and the rates of dispersal and extinction were estimated. Some samples from Genbank data with unknown origin were coded for the entire modern distributional range, otherwise samples were labeled with the known origin of the samples.

Molecular divergence dating was performed in BEAST 1.8 (Drummond et al., 2012) on the XSEDE cluster via the CIPRES Portal (Miller et al., 2010). The BEAST analysis included 71 taxa, with six muroid outgroups, 1 sciuriform outgroup (*Aplodontia rufa*), 11 sciurid outgroups, and 53 nannosciurine taxa, with 1074 bp of cytochrome *b*, 547 bp of *c-myc*, and 531 bp of *RAG-1*. Multiple samples of only cytochrome *b* data were removed when additional individuals representing the same species with complete datasets were present (2 *Callosciurus*, 7 *Sundasciurus*, and 2 *Dremomys*). We used MEGA v5.2.2 to test for a molecular clock using ML values

Table 1

Samples included in the molecular analyses and their collection or publication of origin. GenBank accession numbers for the *c-myc*, *RAG-1* and cytochrome *b* (*cyt b*) sequences for each individual are in the last three columns.

Catalog # or reference	Species	Country	Specific location	<i>RAG-1</i>	<i>c-myc</i>	<i>cyt b</i>
Steppan et al. (2004) and Piaggio et al. (2013)	<i>Aplodontia rufa</i>	N/A	N/A	AY241468	AY239477	JX420007
Steppan et al. (2004) and Jansa et al. (2006)	<i>Batomys granti</i>	N/A	N/A	AY241461	AY239472	DQ191470
NZP 95-322	<i>Callosciurus adamsi</i>	Malaysia	Sabah, Borneo	KP126010	KP126058	KP126035
64664, Oshida et al. (2001)	<i>Callosciurus caniceps</i>	N/A	N/A	N/A	N/A	AB043875
Steppan et al. (2004) and Chang et al. (2010)	<i>Callosciurus erythraeus</i>	N/A	N/A	AY241479	AY239486	HQ698360
USNM 584417	<i>Callosciurus finlaysonii</i>	Myanmar	Mandalay	KP126012	KP126059	KP126037
644236, Oshida et al. (2001)	<i>Callosciurus inornatus</i>	N/A	N/A	N/A	N/A	AB499905
127501, Oshida et al. (2001)	<i>Callosciurus nigrovittatus</i>	N/A	N/A	N/A	N/A	AB043882
64678, Oshida et al. (2001)	<i>Callosciurus notatus</i>	N/A	N/A	N/A	N/A	AB499912
ROM 102139	<i>Callosciurus orestes</i>	Indonesia	East Kalimantan	KP126014	KP126061	KP126036
USNM 583884	<i>Callosciurus phayrei</i>	Myanmar	Mon	KP126013	KP126062	KP126038
MTRH118	<i>Callosciurus prevostii</i>	Malaysia	Sabah, Borneo	KP126032	KP126057	KP126056
Steppan et al. (2004) and Rowe et al. (2008)	<i>Deomys ferrugineus</i>	N/A	N/A	AY241460	AY239471	EU349745
461707, Li et al. (2008)	<i>Dremomys gularis</i>	N/A	N/A	N/A	N/A	EF539337
461708, Li et al. (2008)	<i>Dremomys lokriah</i>	N/A	N/A	N/A	N/A	EF539335
Steppan et al. (2004) and Chang et al. (2010)	<i>Dremomys pernyi</i>	N/A	N/A	AY241482	AY239489	HQ698362
USNM 574326	<i>Dremomys pernyi</i>	China	Sichuan	KP126028	KP126063	KP126039
KIZ 2003012, Li et al. (2008)	<i>Dremomys pernyi</i>	N/A	N/A	N/A	N/A	EF539336
461709, Li et al. (2008)	<i>Dremomys pyrrhomerus</i>	N/A	N/A	N/A	N/A	EF539342
ROM 111385	<i>Dremomys rufigenis</i>	Vietnam	Quang Nam	KP126029	KP126064	KP126040
ROM 111386	<i>Dremomys rufigenis</i>	Vietnam	Quang Nam	KP126030	KP126065	KP126041
KIZ 2004264, Li et al. (2008)	<i>Dremomys rufigenis</i>	N/A	N/A	N/A	N/A	EF539341
ROM 102254	<i>Exilisciurus exilis</i>	Indonesia	East Kalimantan	KP126027	N/A	KP126050
ANU EBU56606	<i>Funambulus pennanti</i>	Australia	Perth (Introduced)	KP126034	KP126081	KP126055
Steppan et al. (2004) and Kruckenhauser et al. (1999)	<i>Marmota monax</i>	N/A	N/A	AY241492	AY239498	AF100719
Steppan et al. (2004), Sudman and Hafner (unpublished)	<i>Microsciurus flaviventer</i>	N/A	N/A	AY241478	AY239485	MFU46169
Steppan et al. (2004) and Galewski et al. (2006)	<i>Microtus irene</i>	N/A	N/A	AY241464	AY239474	AM392370
Steppan et al. (2004) and Conroy and Cook (1999)	<i>Microtus pennsylvanicus</i>	N/A	N/A	AY241463	AY239473	AF119279
Steppan et al. (2004), Bernard et al. (1983) and Hardouin and Tautz (unpublished)	<i>Mus musculus</i>	N/A	N/A	AY241462	AH005318S1	JX945964
Steppan et al. (2004) and Matthee and Robinson (1997)	<i>Paraxerus cepapi</i>	N/A	N/A	AY241498	AY239503	PCU59179
Steppan et al. (2004) and Smith and Patton (1999)	<i>Phyllotis xanthopygus</i>	N/A	N/A	AY241466	AY239475	AF108693
Steppan et al. (2004) and Sudman and Hafner (unpublished)	<i>Sciurillus pusillus</i>	N/A	N/A	AY241471	AY239480	SPU46179
Steppan et al. (2004) and Liu (unpublished)	<i>Sciurotamias davidianus</i>	N/A	N/A	AY241488	AY239495	KC005710
Steppan et al. (2004) and Barber (unpublished)	<i>Sciurus carolinensis</i>	N/A	N/A	AY241475	AY239482	FJ200685
Steppan et al. (2004) and Oshida et al. (2009)	<i>Sciurus stramineus</i>	N/A	N/A	AY241476	AY239484	AB292678
Steppan et al. (2004) and Harrison et al. (2003)	<i>Spermophilopsis leptodactylus</i>	N/A	N/A	AY241487	AY239494	AF157865
BMNH: 1919.11.5.47 Den Tex et al. (2010)	<i>Sundasciurus altitudinus</i>	Indonesia	Sumatra	N/A	N/A	KP120727
USNM 271039, Den Tex et al. (2010)	<i>Sundasciurus altitudinus</i>	Indonesia	Sumatra	N/A	N/A	KP120728
USNM 90570, Den Tex et al. (2010)	<i>Sundasciurus brookei</i>	N/A	N/A	N/A	N/A	KP994921
MTRB7	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126015	KP126067	KP126045
MTRB8	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126016	KP126068	KP126046
MTRB11	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126017	KP126069	KP126047
MTRB12	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126018	KP126070	KP126049
MTRB15	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126019	KP126071	KP126048
MTRH151_Kinabalu	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126020	KP126076	KP126051
MTRH163_Kinabalu	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126022	KP126079	KP126052
MTRH168_Kinabalu	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126023	KP126078	N/A
MTRH172_Kinabalu	<i>Sundasciurus everetti</i>	Malaysia	Sabah, Borneo	KP126021	KP126077	KP126053
USNM 121628, Den Tex et al. (2010)	<i>Sundasciurus fraterculus</i>	Indonesia	South Pagi Island	N/A	N/A	KP120730
USNM 311463, Den Tex et al. (2010)	<i>Sundasciurus hippurus</i>	Malaysia	Sarawak, Borneo	N/A	N/A	KP120733
USNM 141032, Den Tex et al. (2010)	<i>Sundasciurus hippurus</i>	Indonesia	Sumatra	N/A	N/A	KP120732
USNM 142273, Den Tex et al. (2010)	<i>Sundasciurus hippurus</i>	Indonesia	Sumatra	N/A	N/A	KP120734
USNM 488406, Den Tex et al. (2010)	<i>Sundasciurus hippurus</i>	Malaysia	Selangor	N/A	N/A	KP120731
USNM 477851, Den Tex et al. (2010)	<i>Sundasciurus hoogstraali</i>	N/A	N/A	N/A	N/A	KP120735
USNM 292576, Den Tex et al. (2010)	<i>Sundasciurus jentinki</i>	Malaysia	Sabah, Borneo	N/A	N/A	KP120736
MTRH 117	<i>Sundasciurus jentinki</i>	Malaysia	Sabah, Borneo	KP126033	KP126080	KP126054
USNM 477866, Den Tex et al. (2010)	<i>Sundasciurus juvenus</i>	Philippines	N/A	N/A	N/A	KP120737
FMNH 196005 Sp_DSB5065, Den Tex et al. (2010)	<i>Sundasciurus juvenus</i>	Philippines	N/A	N/A	N/A	KP120738
FMNH 195288, Den Tex et al. (2010)	<i>Sundasciurus juvenus</i>	Philippines	N/A	N/A	N/A	KP120739
ROM 102098, Den Tex et al. (2010)	<i>Sundasciurus lowii</i>	Indonesia	East Kalimantan, Borneo	N/A	N/A	KP120742
USNM 488447, Den Tex et al. (2010)	<i>Sundasciurus lowii</i>	Malaysia	Selangor	N/A	N/A	KP120740
MTRB17	<i>Sundasciurus lowii</i>	Malaysia	Sabah, Borneo	KP126024	KP126074	KP126042
NZP 93-434	<i>Sundasciurus lowii</i>	Malaysia	Sabah, Borneo	KP126025	KP126072	KP126043
NZP 96-560	<i>Sundasciurus lowii</i>	Malaysia	Sabah, Borneo	KP126026	KP126073	KP126044
USNM 462199, Den Tex et al. (2010)	<i>Sundasciurus mindanensis</i>	Philippines	N/A	N/A	N/A	KP120743
USNM 477947, Den Tex et al. (2010)	<i>Sundasciurus moellendorfi</i>	Philippines	N/A	N/A	N/A	KP120744
FMNH 191420, Den Tex et al. (2010)	<i>Sundasciurus phillipinensis</i>	Philippines	N/A	N/A	N/A	KP120746

(continued on next page)

Table 1 (continued)

Catalog # or reference	Species	Country	Specific location	RAG-1	c-myc	cyt b
FMNH 194781, Den Tex et al. (2010)	<i>Sundasciurus philippinensis</i>	Philippines	N/A	N/A	N/A	KP120745
FMNH 195285, Den Tex et al. (2010)	<i>Sundasciurus rabori</i>	Philippines	N/A	N/A	N/A	KP120747
EAR1306, Den Tex et al. (2010)	<i>Sundasciurus samarensis</i>	Philippines	N/A	N/A	N/A	KP120748
USNM 458736, Den Tex et al. (2010)	<i>Sundasciurus samarensis</i>	Philippines	N/A	N/A	N/A	KP120749
USNM 477948, Den Tex et al. (2010)	<i>Sundasciurus steeri</i>	Philippines	N/A	N/A	N/A	KP120750
USNM 87085, Den Tex et al. (2010)	<i>Sundasciurus tahan</i>	N/A	N/A	N/A	N/A	KP994922
ROM 102157, Den Tex et al. (2010)	<i>Sundasciurus tenuis</i>	Indonesia	East Kalimantan, Borneo	KP126031	KP126075	KP120754
USNM 488459, Den Tex et al. (2010)	<i>Sundasciurus tenuis</i>	Malaysia	Johor	N/A	N/A	KP120751
USNM 488458, Den Tex et al. (2010)	<i>Sundasciurus tenuis</i>	Malaysia	Johor	N/A	N/A	KP120752
Nunome et al. (2007), Steppan et al. (2004) and Obolenskaya et al. (2009)	<i>Tamias sibiricus</i>	N/A	N/A	AB253978	AY239496	FJ655250
Steppan et al. (2004) and Barber (unpublished)	<i>Tamiasciurus hudsonicus</i>	N/A	N/A	AY241474	AY239482	FJ200685
Steppan et al. (2004) and Li et al. (2008)	<i>Tamias swinhoei</i>	N/A	N/A	AY241481	AY239488	EF539334
Steppan et al. (2004) and Herron et al. (2005)	<i>Xerus inauris</i>	N/A	N/A	AY241485	AY239492	DQ010376

Table 2

Average external proportions from representatives of plain long-nosed squirrels and Sunda squirrels measured in this study. Abbreviations: n, number of individuals from which data were collected; HB, head-body length; Tail, tail length; T/HB proportion of tail to HB; CL, condylobasal length; NL, nasal length; NL/CL proportion of nasal length to skull size. All measurements in mm. The BMGS (*Dremomys everetti*) is shown in bold.

Species	n	HB	Tail	T/HB	SD (range)	CL	NL	CL/NL	SD (range)
<i>Dremomys everetti</i>	18	162.30	95.7	0.59	0.09 (0.35–0.73)	39.2	14.1	0.36	0.03 (0.31–0.47)
<i>Dremomys lokriah</i>	7	190.0	127.1	0.67	0.07 (0.57–0.77)	42.2	15.3	0.36	0.02 (0.34–0.41)
<i>Dremomys pernyi</i>	10	186.3	138.0	0.73	0.13 (0.64–0.88)	44.8	16.4	0.35	0.01 (0.36–0.38)
<i>Dremomys rufigenis</i>	23	186.0	145.7	0.79	0.11 (0.52–1)	47.7	17.1	0.36	0.03 (0.28–0.42)
<i>Dremomys pyrrhomeus</i>	3	218.7	158.3	0.72	0.08 (0.64–0.78)	50.2	16.7	0.36	0.02 (0.33–0.38)
<i>Dremomys gularis</i>	1	235.0	162.0	0.69	N/A (–)	49.4	20.1	0.41	N/A (–)
<i>Sundasciurus brookei</i>	6	159.6	136.2	0.86	0.16 (0.66–1.03)	37.4	12.6	0.34	0.03 (0.31–0.41)
<i>Sundasciurus hippurus</i>	16	234.9	255.1	1.09	0.11 (0.85–1.31)	51.9	16.8	0.32	0.01 (0.29–0.34)
<i>Sundasciurus jentinki</i>	12	127.1	116.2	0.92	0.08 (0.79–1.06)	31.1	10.3	0.33	0.02 (0.30–0.35)
<i>Sundasciurus tenuis</i>	7	136.3	115.7	0.85	0.03 (0.77–0.88)	31.7	10.3	0.32	0.02 (0.30–0.35)
<i>Sundasciurus lowii</i>	11	127.3	86.5	0.68	0.06 (0.53–0.75)	32.9	10.4	0.32	0.01 (0.30–0.33)

under the Hasegawa–Kishino–Yano (1985) model. The results rejected the null hypothesis, and so a relaxed lognormal clock was used for each partition in BEAST 1.8. We used a Yule process of speciation, and three lognormal calibration points were defined based on the fossil record: (1) 40 MYA (mean: 36.6; 95% quantiles: 28.8–46.5) for the root of Sciuridae, which has been previously used for dating estimates (Eldredge and Stanley, 1984; Mercet and Roth, 2003). All Sciurid taxa were included in this calibration, with the muroid outgroups outside of this taxa panel. (2) 24 MYA (mean: 23.9; 95% quantiles: 20.2–28.2) for the split within muroid rodent outgroups, which has been used in previous murine phylogenies (Steppan et al., 2004); and (3) 14 MYA (mean: 14.4; 95% quantiles: 7.4–30.1) for the subfamily Nannosciurinae, which included 60 individuals from three genera (*Sundasciurus*, *Callosciurus* and *Dremomys*) (Qiu, 2002). For all node calibration points, the fossil age was coincident with the median of the lognormal distribution. The evolution rates for each partition were estimated using the lognormal relaxed clock with the operators left to default. Four replicates of 100 million chains were run and evaluated for convergence. An empty alignment was run to evaluate the effect of the priors on the dataset (the ESS values and support from the empty alignment should be different from the full analysis, to determine how much of an effect the priors had on the results). Tracer 1.5 was used to evaluate the convergence of the BEAST runs by assessing the Effective Sample Size (ESS) values, and Tree annotator was used to generate a consensus tree. FigTree v1.4.1 (Rambaut and Drummond, 2012) was used to generate the molecular time tree.

2.3. Morphological analyses

2.3.1. Material

One hundred and twenty-five specimens from 12 species, including all recognized species of plain long-nosed squirrels

(Corbet and Hill, 1992; Thorington and Hoffmann, 2005), were used for morphological analyses (Table S1). In morphometric comparisons, only adults were selected, as determined by closure of the basilar synchondrosis and eruption of all teeth. These squirrels are not sexually dimorphic (Tenzin et al., 2013; Moore and Tate, 1965), and both sexes were included in our analyses. Sixteen cranial measurements (as described in Hayashida et al., 2007) were taken from all six species of plain long-nosed squirrels and six species of Sunda squirrels with digital calipers graduated to 0.1 mm. A total of 92 specimens were used for the quantitative traits, and 115 specimens for qualitative traits (Table S1).

2.3.2. Other craniodental morphology

For craniodental characters, we examined essentially all specimens of Sunda and plain long-nosed squirrels stored at USNM (~750 specimens). Cranial characters used by other authors to distinguish the two genera (cf. Corbet and Hill, 1992; Moore, 1959, 1958; Moore and Tate, 1965) include the relative length of the rostrum (longer in *Dremomys*, shorter in *Sundasciurus*), the relative size and position of the upper premolars, P³ and P⁴ (P³ regarded as small relative to, and situated lateral to, P⁴, in *Dremomys*, and larger and more directly anteriorly placed relative to P⁴ in *Sundasciurus*: Corbet and Hill, 1992) and the morphology of the auditory bulla (Corbet and Hill, 1992; Moore, 1959, 1958; Moore and Tate, 1965), with the “bullar septae Y-shaped, forming anterior-mesial lobe” (Corbet and Hill, 1992) in Sunda but not in plain long-nosed squirrels. We also documented the presence or absence of the temporal foramen (characteristic of plain long-nosed squirrels, often absent in Sunda squirrels; Moore and Tate, 1965). We evaluated relative rostral length by comparing the length of the nasal bones relative to the condylobasal length of the skull (Tables 2 and 3).

Table 3

Characterization of discrete characters previously used to discriminate between the plain long-nosed squirrels and Sunda squirrels. Characterization of the Bornean mountain ground squirrel, the plain long-nosed squirrels (as represented by *Dremomys pernyi*, the type species) and the Sunda squirrels (as represented by *Sundasciurus lowii*, the type species).

Character	<i>Dremomys</i>	" <i>Dremomys</i> " <i>everetti</i>	<i>Sundasciurus</i>
Temporal fossa	Present (small)	Absent	Absent
Anteromedial expansion of auditory bulla	Not pronounced	Intermediate	Pronounced
P3/P4 size/position	Inconclusive	Inconclusive	Inconclusive
Rostral length	Long	Long	Short
Tail length	Generally short	Short	Short to long

1. Moore and Tate (1965), 2. Corbet and Hill (1992).

2.3.3. External proportions

The plain long-nosed squirrels are montane, ground-dwelling squirrels generally characterized by having a relatively short tail compared to other Southeast Asian squirrel genera, including the Sunda squirrels (Corbet and Hill, 1992; Moore and Tate, 1965). We evaluated this aspect in specimens by comparing the length of the tail relative to the head-body length. External measurements were taken from specimen tags as measured by the original collectors. Tail length was subtracted from total length to calculate head-body length, if not explicitly given on the specimen tag. A total of 115 specimens were included in these comparisons (Table S1).

2.3.4. Analysis of morphological data

RStudio v 0.98.1062 was used to generate a Principal Component Analysis (PCA) for morphometric comparisons of the cranial measurements, and to compare external and cranial proportions for the BMGS to a range of species of plain long-nosed and Sunda squirrels, especially Perny's long-nosed squirrel (*D. pernyi*) and Low's squirrel (*S. lowii*) (the respective type species of *Dremomys* and *Sundasciurus*). Measurements were transformed to natural logarithms for the PCA. A multivariate analysis of variance (MANOVA) was done on the same cranial measurements as detailed above (also natural logarithm transformed). A Wilks lambda, Pillar's trace and Hotelling–Jawley trace variant of the MANOVA were calculated.

In addition to standard morphometric analysis, we performed a phenotypic trajectory analysis (PTA) (Adams and Collyer, 2009), with the R package 'geomorph' to calculate the phenotypic trajectories for a subset of species included in the morphological analysis. Species included in this analysis were *Sundasciurus lowii*, *S. hippurus*, *S. tenuis*, *S. everetti*, *Dremomys pernyi*, and *D. rufigenis*. Data input to geomorph were the log transformed values from the PCA, with PC1 and PC2 used for the first PTA, and a second comparison performed with PC2 and PC3 to evaluate the effect of PC1 (which accounted for some 98% of the observed variation). We also tested the effect of using individual log transformed cranial measurements, with similar results (data not shown). Each replicate was visualized after 1000 iterations.

3. Results

3.1. Molecular phylogenetic analysis

Cytochrome *b* (1074 bp), *c-myc* (548 bp), and *RAG-1* (533 bp) sequences were generated from all 24 tissue samples (Table 1). *RAG-1* contained 25 parsimony informative (PI) sites for alignments containing *Dremomys* + *Sundasciurus* and 8 for *Dremomys* alone (not including the BMGS). *C-myc* contained 9 PI sites for the *Dremomys* + *Sundasciurus* alignment, and 2 for *Dremomys* alone. All newly generated sequences have been uploaded to Genbank (accession numbers in Table 1). All three genes generated congruent tree topologies, the two nuclear genes generating moderate support alone (data not shown), and strong support when

concatenated with mitochondrial data. The most parsimonious tree (of the concatenated dataset) had a tree length of 5982 steps (CI = 0.218; RI = 0.512; and RC = 0.112). The topology of the single ML tree was concordant with the MP results ($\kappa = 3.94$, $\ln = -30655.86$). The topology of the BI tree was also concordant, and was more highly resolved than either the MP or ML analyses, with several well supported nodes (Fig. 2).

The relationship between the sequences reported in Li et al. (2008) was recovered, but the addition of more individuals resulted in the polyphyly of the red-cheeked squirrels (*D. rufigenis*) with high support (BI 1 and MP and ML bootstrap support >90). The Vietnam red-cheeked squirrels were more closely related to the red-throated squirrel (*D. gularis*), and the animals from southern China were more closely related to the red-hipped squirrel (*D. pyrrhomerus*) (Fig. 2). The sequences included from Li et al. (2008) are only mtDNA data, but were used to include representatives of all species of *Dremomys*.

All species of plain long-nosed squirrels formed a monophyletic clade with high support (BI posterior probability of 1 and ML >90) except the BMGS. This species was nested within a clade containing members of Sunda squirrels, with high support (BI posterior probability of 0.99 and ML bootstrap of 100). Within the Sunda squirrels, the BMGS clustered within the subgenus *Aletesciurus* (Moore, 1958), with high support (BI 0.98). The subgenus *Sundasciurus* was not recovered as monophyletic, as in Den Tex et al. (2010).

3.2. Molecular dating and ancestral range reconstruction

The common ancestor of the BMGS and the Sunda squirrels diverged from the lineage leading to the plain long-nosed squirrels approximately 11.61 MYA (95% HPD 9.1–14.56) (Fig. 3). The divergence between the BMGS and its closest relatives, which are Sunda squirrels, was estimated at 6.62 MYA (95% HPD 4.93–8.59). The most recent common ancestor for all plain long-nosed squirrels except the BMGS was estimated at 5.97 MYA (95% HPD 4.34–7.95), and the common ancestor of all Sunda squirrels including the BMGS was 8.34 MYA (95% HPD 6.41–10.48). Within plain long-nosed squirrels (except the BMGS), the most recent speciation event was dated to 2.9 MYA (95% HPD 1.77–4.17, between the Chinese red-cheeked squirrel and the red throated squirrel) and the average age of all of the species was 4.4 MYA (range 5.97–2.9 MYA). Within *Sundasciurus* (here including the BMGS), the oldest split within the genus was the BMGS (6.62 MYA) with the most recent speciation events in the Philippines during the Pleistocene (~170,000 years ago). The empty alignment recovered a poorly supported tree, with low ESS values, indicating the priors were not affecting the results, rather the data drove the topology and support observed.

The output from LaGrange recovered ancestral ranges with a maximum of three regions per reconstructed node (see Fig. S1). The distribution shown on the ML tree corresponds to the common ancestor of each labeled node. The real probability was at least 85%

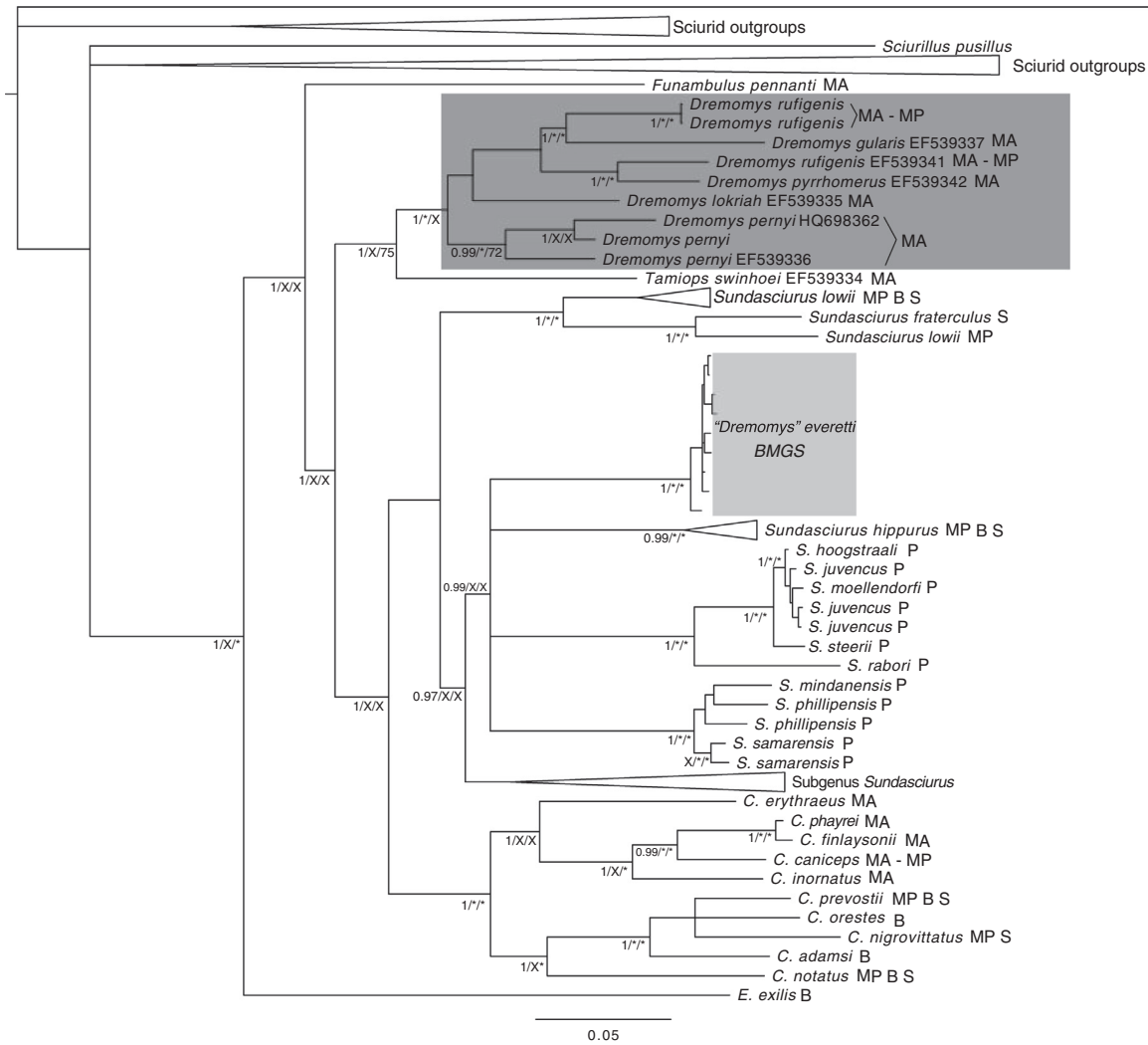


Fig. 2. Bayesian phylogeny of all species of plain long-nosed squirrels, and a variety of nannosciurine species, based on two nuclear and one mitochondrial gene sequences. Support values are abbreviated with BI first, and MP and ML values are abbreviated with a * when bootstrap support was above 80. Nodes with poor support, or where certain methods generated a polytomy, are indicated with an X. The geographic range of each species is indicated with the following abbreviations following the species, MA = mainland Asia, MP = Malay Peninsula, S = Sumatra, B = Borneo, P = Philippines. The BMGS is not labeled, but distributed solely in Borneo.

for each node. No nodes were supported with less than 85% support. The reconstruction revealed multiple movements from mainland Asia into Borneo, at least four times in the taxa evaluated here (*Exilisciurus*, *Callosciurus*, and at least twice from the *Sundasciurus*, across both recognized subgenera). It appears the clade containing the BMGS moved into Borneo with the most closely related extant taxa distributed in Asia. Our results also suggest that the Malay Peninsula has also been colonized at least five times (by: *Dremomys rufigenis*, *Sundasciurus lowii*, *Sundasciurus tenuistahan*, *Sundasciurus hippurus*, and *Callosciurus nigrovittatus*). The Philippines appear to have been colonized twice by squirrels.

3.3. Morphological taxonomy

Sixteen cranial measurements were taken from 92 individuals representing a variety of species, including all recognized plain long-nosed squirrel species, six species of Sunda squirrels from Borneo and one from the Philippines. The PCA recovered the plain long-nosed squirrels as a distinct cluster, and the Sunda squirrels as two separate clusters. The BMGS fell between the two genera (Fig. S2). The largest bodied species of Sunda squirrels measured, *Sundasciurus hippurus* and *S. philippensis*, comprised a cluster

separate from the other squirrels of both genera. In the PCA, 91.78% of the variance was explained by PC1 alone (a component primarily reflecting overall size), and 2.57% explained by PC2 (Table S2). When PC2 and PC3 were plotted no additional resolution was visualized (data not shown). The MANOVA revealed significant differences between all species compared (*Dremomys pernyi*, *Sundasciurus lowii* and the BMGS; Table S3).

The plain long-nosed squirrels have relatively long rostra and short tails, whereas the Sunda squirrels have shorter rostra and variable tail lengths (Table 2, Figs. 4, S3 and S4). The BMGS had more variation in tail and rostrum length than mainland plain long-nosed squirrel species (Fig. S3), thus falling morphologically intermediate between the two genera with respect to these characters. Within the Sunda squirrels, rostral length relative to skull length was longer in the montane species (*Sundasciurus brookei* and *S. jentinki*) than in lowland species (Table 2). Other characters that have historically been used to discriminate the genera were inconclusive (Table 3). The anteromedial expansion of the auditory bulla of the BMGS was generally intermediate between species of both genera (Fig. 4), and showed considerable variation, generally in the morphological direction of the Sunda squirrels (Moore and Tate, 1965:291). Positional and relative size variation in the upper

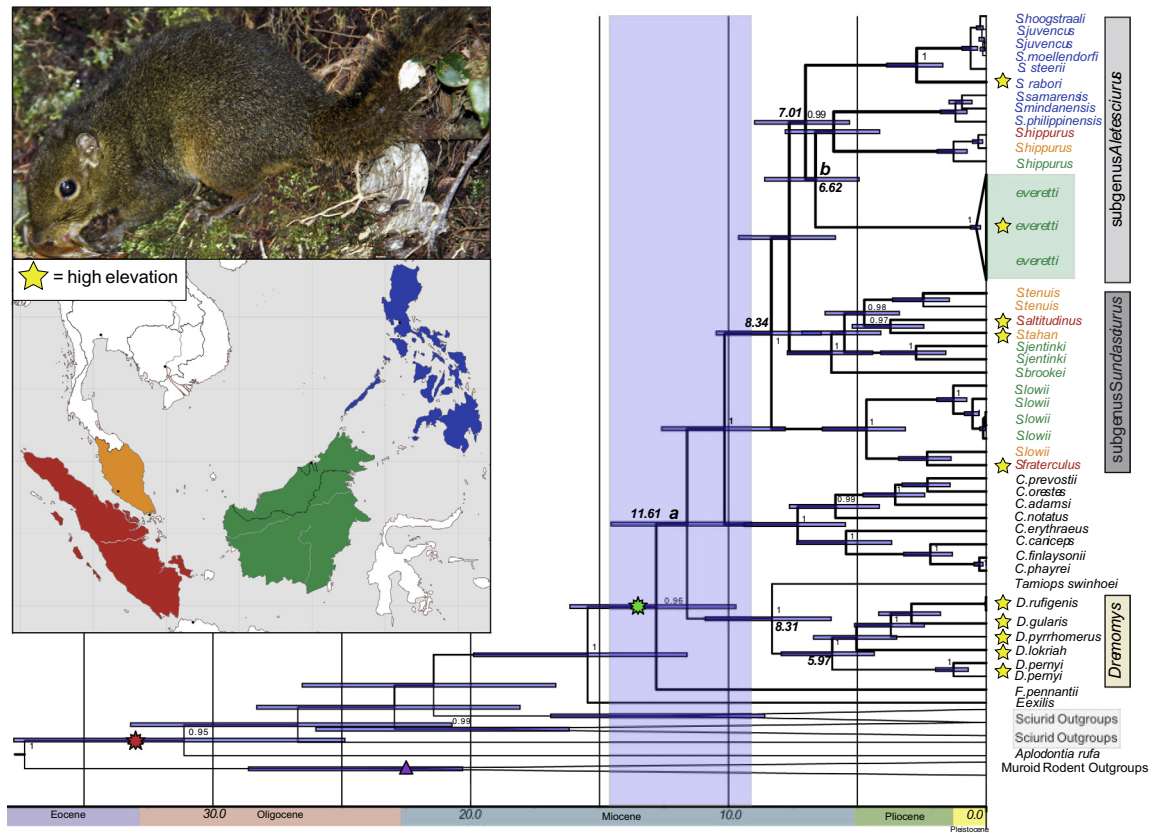


Fig. 3. Dated phylogeny including all species of plain long-nosed squirrels and 16 of the 17 Sunda squirrels. Dates are indicated on the bar at the bottom of the figure in millions of years before present. Node 'a' is the common ancestor for plain long-nosed and Sunda squirrels, and is estimated to be 11.61 MYA (confidence interval highlighted in blue). Node "b" shows the split within the Sunda squirrels to the Bornean mountain ground squirrels (BMGS) estimated to be 6.62 MYA. Species are colored to match the inlaid map, representative of species distribution, and the BMGS is highlighted in green. A geologic timescale is portrayed at the bottom of the tree. The two subgenera of Sunda squirrels are highlighted with gray boxes. All high elevation endemics are marked with a yellow star. The three fossil calibration points are portrayed as the purple triangle (calibration #2, Murid rodents, 24 MYA) and the red and green stars (#1 root of squirrels, 36 MYA, #3 and "Nannosciurine" 14 MYA calibration point). The photograph above the inlaid map is a BMGS from Kinabalu Park, Sabah, Malaysia.

premolars, regarded as a distinguishing feature between plain long-nosed and Sunda squirrels by Corbet and Hill (1992), was sufficiently variable across the specimens that we did not find it useful to distinguish them. One discrete character that distinguishes the BMGS from the plain long-nosed squirrels is the presence/absence of the temporal foramen (Fig. 4), which, as Moore and Tate (1965: 291) observed, is absent in the BMGS but consistently present (albeit usually very small) in other species of plain long-nosed squirrels. Close examination of specimens at USNM revealed that the temporal foramen is variably present in the majority of Sunda squirrels, but is absent (or present at very low frequency) in several species, especially in those taxa most closely related to the BMGS (*Sundasciurus hippurus*, *S. mindanensis*, *S. samarensis*) and in one other montane endemic (*S. jentinki*). The absence of the temporal foramen in the BMGS is thus a character that is more consistent with its classification as a Sunda squirrel (*Sundasciurus*) than as a plain long-nosed squirrel (*Dremomys*).

3.4. Morphological convergence

In addition to the observation of shared characters such as longer rostral length relative to skull length in the montane species than in lowland species (Table 2), phenotypic trajectory analysis performed using the R package 'geomorph' indicated convergence of BMGS with the high elevation plain long-nosed squirrels (Fig. 5). This analysis shows two trajectories, one which represents the plain long-nosed squirrels, and another for the Sunda squirrels

(here including the BMGS), and the points which represent the BMGS have a strong directional pull into the phenotypic space of the plain long-nosed squirrels, providing evidence of phenotypic convergence (see Fig. 5A). When PC2 and PC3 were run on a simplified dataset of only *Dremomys pernyi*, the BMGS and *Sundasciurus lowii* the pattern remained the same, with the amount of phenotypic change between the BMGS and *Sundasciurus lowii* greater than when comparing the BMGS to *Dremomys pernyi*, implying greater phenotypic difference between the BMGS and the *Sundasciurus* than the *Dremomys* (see Fig. 5B). Regardless of how we ran the program we recovered similar results, with the BMGS appearing more phenotypically similar to the other species of *Dremomys* than the Sunda squirrels (including individual morphometric measurements, data not shown).

4. Discussion

4.1. Taxonomic implications

The polyphyly of the genus of plain long-nosed squirrels caused by the BMGS requires revision of this species (Fig. 2). Both mitochondrial and nuclear DNA supported the placement of the BMGS within the Sunda squirrels. This result is consistent with the biogeography of these generic lineages: the Sunda squirrels are distributed throughout Sundaland (and into the Philippines), and the BMGS is an endemic Sundaic species; while the other plain long-nosed squirrels are restricted to mainland Asia. A number of

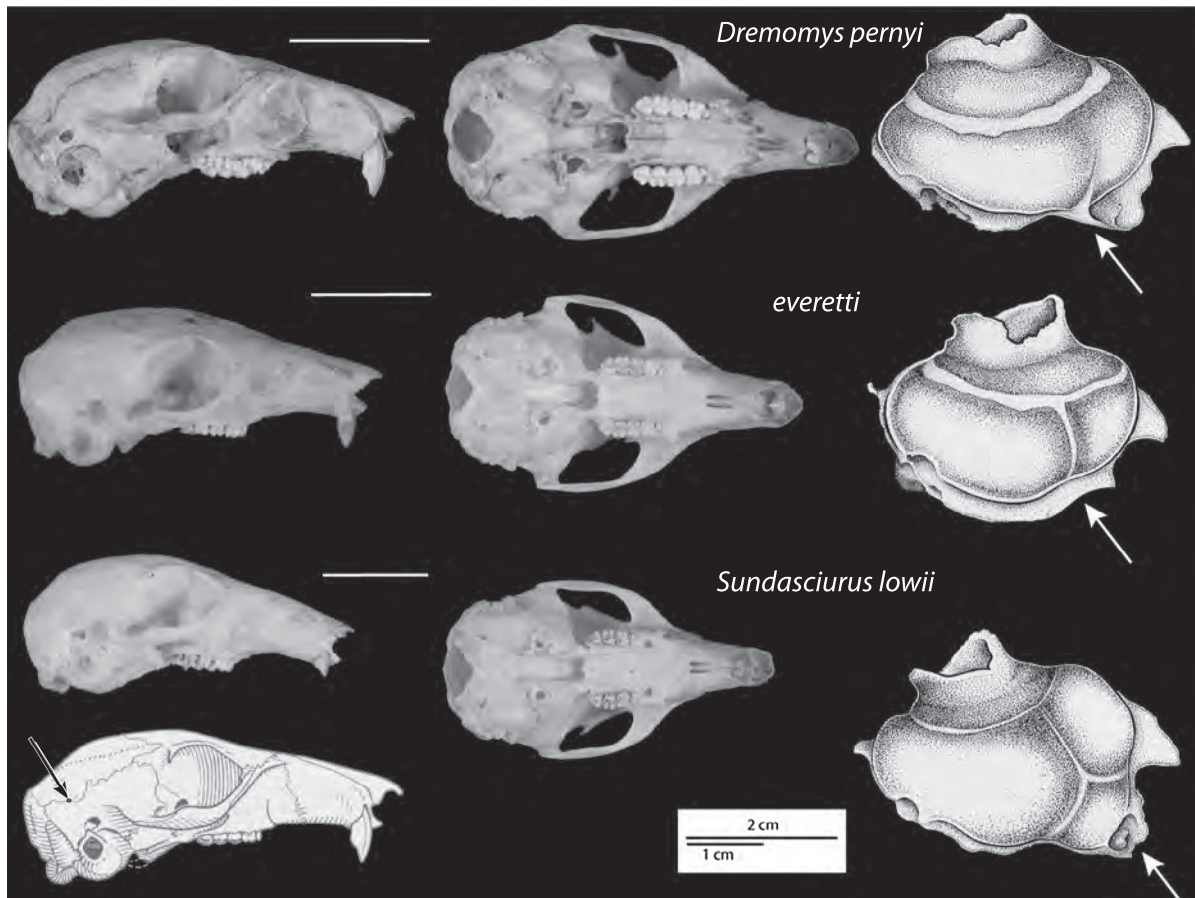


Fig. 4. Comparative photographs of skulls illustrating qualitative cranial characters previously suggested to differentiate the plain long-nosed squirrels from the Sunda squirrels. The type species of *Dremomys* is Perny's long-nosed squirrel (*Dremomys pernyi*), represented by USNM 256110, and the type species of *Sundasciurus* is Low's squirrel (*Sundasciurus lowii*), represented by USNM 197276. The Bornean mountain ground squirrel ("*Dremomys*" *everetti*) is represented by USNM 292628. The white bars highlight the length of the nasal bones in each individual, and the scale bar is for the photographs. In the bottom left is an illustration of a cranium showing the temporal foramen, indicated by the black arrow (modified from Moore and Tate, 1965). Line drawings of the auditory bullae are provided to the right of each skull to clearly illustrate the characters described in the text, and the white arrows highlight the differences observed in the expansion of the third chamber of the auditory bulla between the three species included.

morphological traits, including body size, rostral length, the morphology of the auditory bulla, and the absence of a temporal foramen in the cranium, also support the recognition of the BMGS as a specialized montane representative of *Sundasciurus* rather than a member of the genus *Dremomys* (Table 3). Accordingly, we formally re-classify the species here as *Sundasciurus everetti*. This taxonomic change builds upon previously published observations and impressions that the BMGS was the most divergent species of plain long-nosed squirrel (Corbet and Hill, 1992; Moore and Tate, 1965), and resolves longstanding confusion as to the true generic affinities of the species (e.g. Moore and Tate, 1965; Allen and Coolidge, 1940; Banks, 1933). Presciently, Moore and Tate (1965: 291) previously discussed similarities between the BMGS and the subgenus *Aletesciurus* of *Sundasciurus* in the morphology of the auditory bulla, foreshadowing this transfer.

The Sunda squirrels (*Sundasciurus*) have been traditionally subdivided into two subgenera, *Aletesciurus* and *Sundasciurus* (Moore, 1958). *Aletesciurus* contains one widely distributed species (*Sundasciurus hippurus*) of the Malay Peninsula, Sumatra, and Borneo), and nine species distributed in the Philippines (*S. samarensis*, *S. philippinensis*, *S. mindanensis*, *S. rabori*, *S. steerii*, *S. moellendorffi*, *S. davensis*, *S. juvencus*, and *S. hoogstraali*). The subgenus *Sundasciurus* includes seven species (*Sundasciurus altitudinis*, *S. brookei*, *S. fraterculus*, *S. jentinki*, *S. lowii*, *S. tahan*, and *S. tenuis*), all distributed in Sundaland (see Fig. 1; Den Tex et al., 2010;

Thorington and Hoffmann, 2005; Thorington et al., 2012). Our phylogeny suggests that the Sunda squirrels are made up of 5–6 major phylogenetic lineages of considerable time-depth, which correspond imperfectly to previously recognized subgenera. The subgenus *Aletesciurus* is monophyletic (including BMGS), but the subgenus *Sundasciurus* was not recovered as monophyletic (as in Den Tex et al., 2010). For this reason, we suggest suspending the traditional classification of *Sundasciurus* into two subgenera. A more detailed review in the future may show that the various deep lineages or species-groups recovered within *Sundasciurus* are better recognized as distinct subgenera.

The apparently strong selective forces that drove the convergence between the BMGS and plain long-nosed squirrels may also confound the delineation of other species within the plain long-nosed squirrels. The deep polyphyly in the red-cheeked squirrel may indicate that this species should be split into multiple species. The animals included in this phylogeny are from Vietnam and south China. In their morphological study, Endo et al. (2003) found distinct morphological differentiation between northern and southern red-cheeked squirrels. Both China and Vietnam would be in one of these morphological groups, possibly indicating that this species should be split into at least three species. Studies on morphological variation in the other widespread species, Perny's long-nosed squirrel, also found differentiation between four taxonomic units they referred to as subspecies (Song, 2008). Neither

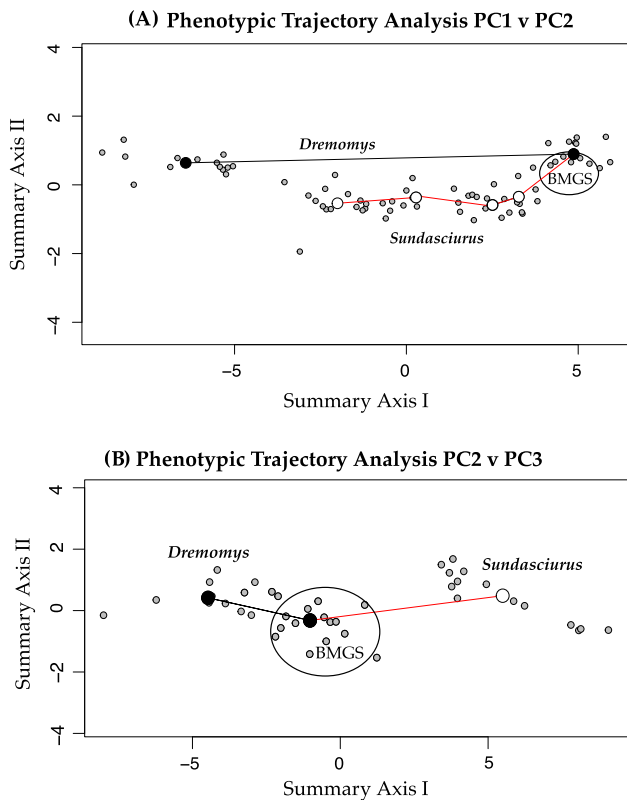


Fig. 5. The phenotypic trajectory analysis (PTA) of the plain long-nosed squirrels (*Dremomys*) and several species of Sunda squirrels (*Sundasciurus*). (A) PTA based on PC1 and PC2 of the PCA including two species of *Dremomys* (left black dot = average for *D. rufigenis*, right black dot = average for *D. pernyi*), with small gray dots representing individuals. The open white circles on the bottom line represent the phenotypic average of five species of Sunda squirrels, from left to right, *Sundasciurus lowii*, *S. jentinki*, *S. tenuis*, and *S. hippurus*. Again the gray points represent individuals. The BMGS is the final set of points, which pulls sharply towards the phenotypic space of *D. pernyi*. The black line connects the two species of *Dremomys*, and the red line connects the *Sundasciurus*. (B) Simplified PTA of only *D. pernyi*, the BMGS and *S. lowii* evaluated for PC2 and PC3 of PCA analysis to visualize the effect of PC1. Here *D. pernyi* is the cluster of gray dots and the average shown as the large black dot on the left side of the figure, with the BMGS shown in the center black dot (average) and surrounding gray points representing all individuals. *Sundasciurus lowii* is shown averaged in the open white circle, with each individual point shown in gray. The amount of change in phenotypic space is far less between the BMGS and the *Dremomys* here when compared to *S. lowii*. The combination of both PTA's show that the BMGS has more phenotypic similarity to the *Dremomys* than to the *Sundasciurus*, the genus with whom it is most phylogenetically closely related. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

of these morphological studies was able to survey the entire distribution of the target species, and important localities such as Taiwan and Hainan were not included. Further study of the genetic diversity across the range of the recognized species may uncover undescribed species, as was found for the sister genus *Tamiops* (Chang et al., 2011).

4.2. Convergent evolution

The highest mountains of Borneo are situated in the north of the island, comprising a range spanning over 350 km in length. Within this mountain chain, Mount Kinabalu is the tallest peak, reaching 4101 m. These uplands constitute a major zone of endemism within Borneo, and the BMGS is one of 22 mammal species endemic to this mountain (Medway, 1977; Payne et al., 1985). Our phylogeny reveals that the BMGS represents one of four independently evolved lineages of montane endemics (species

primarily distributed above 1000 m) within the Sunda squirrels. The other three examples are *Sundasciurus rabori*, endemic to the mountains of Palawan (Heaney, 1979; Heaney et al., 1998); the sister species-pair *S. altitudinus* and *S. tahan*, endemic to the mountain chains of Sumatra and the Malay Peninsula, respectively (Den Tex et al., 2010); and *S. jentinki*, also a Bornean montane endemic (Den Tex et al., 2010; Payne et al., 1985), *S. brookei* is also endemic to the Bornean mountains, but predominates in hill forests at somewhat lower elevations). Some of the traits that have been suggested to define the plain long-nosed squirrels, such as longer rostral length, shorter tail, and dark dense pelage, also characterize high elevation Sunda squirrels. This suggests that these characters are the result of convergent evolution in similar habitats (high elevation tropical forest) as opposed to phylogenetically informative traits. This is supported by the phenotypic trajectory analysis (Fig. 5), which showed the BMGS had more phenotypic similarity to the long-nosed squirrels (*Dremomys*) than the Sunda squirrels (*Sundasciurus*). Although the precise mechanism by which selection acts on these traits is not obvious, these traits also characterize other much more distantly related small mammals that share this high elevation habitat, such as the mountain and smooth-tailed treeshrews (*Tupaia montana*, *Dendrogale melanura*), and lesser gymnure (*Hylomys suillus dorsalis*) (Payne et al., 2007), suggesting that they may be adaptive to the montane environment. While this may reflect only coincidence, it remains a largely unstudied area in elevational adaptations, with most research currently focusing on high elevation hypoxia (Storz et al., 2010).

4.3. Biogeographical implications

Studies of the biogeography and paleoenvironments of the Sunda Shelf have suggested that during times of low sea level stands, many islands within the Sunda Shelf were connected by forested environments (Cannon et al., 2009; Leonard et al., 2015; Raes et al., 2014; Woodruff, 2010). This geographic history may have been important in producing the remarkable biodiversity observed in Sundaland today (de Bruyn et al., 2014; Leonard et al., 2015), and for the colonization of the Philippines by Sunda squirrels (Fig. 3). Other geological processes, such as volcanic activity and uplifting may also have influenced this biodiversity on a longer time scale (Esselstyn et al., 2009; Heaney, 2000; Jansa et al., 2006). These geological and environmental factors may affect speciation of lowland and highland species differently due to different levels of habitat stability, distribution and connectivity (Cannon et al., 2009; Haq et al., 1987).

During the Miocene the sea level and climate fluctuated greatly (Haq et al., 1987), and likely allowed for the expansion of montane habitat into areas which are presently dominated by lowland forest. This has been modeled for the Pleistocene, but not as far back as the Miocene (Cannon et al., 2009). The expansion of the montane forests during this time period may have allowed for the movement and diversification of montane lineages when additional suitable habitat was available. The lowest sea level has been estimated around 10 MYA, with less dramatic shifts occurring throughout the Miocene, followed by very dramatic fluctuations during the Pleistocene (Haq et al., 1987). The effect of sea level change in Sundaland has likely been significant, with periods of low sea level connecting the various landmasses together, followed by isolation when sea levels rise (see Leonard et al., 2015 for patterns in vertebrate colonization through time). The BMGS diverged from other species of *Sundasciurus* around 6.6 MYA, which is near a time of low sea level circa 6 MYA (see Mercer and Roth Fig. 3, and Haq et al., 1987), and likely implies the diversification events between the BMGS, the *S. hippurus* clade, and possibly the *S. tenuis* and *S. lowii* clades all diversified following this time of low sea level. While advances have been made in biogeographic modeling

(in order to better represent real world examples) complex biogeographical systems such as those that occurred in Sundaland, with repeated waves of colonization and extinction, remain beyond the capacity of these models (Borregaard et al., 2015; Heaney et al., 2013). A model incorporating sea level change through time, may be able to more accurately model scenarios such as this.

High elevation habitats are currently at their minimum extent, and were much more widely distributed throughout most of the Pleistocene, approximately the last two million years (Cannon et al., 2009; Woodruff, 2010). All of these high elevation endemic squirrels appear to be strongly limited in their distribution by environmental factors. The low level of genetic variation identified when multiple individuals from the same region were sampled could suggest that these populations have endured small effective population sizes over long periods of time (Frankham, 1996). This potentially places the populations inhabiting these habitats at increased risk of extinction. Additional habitat perturbation could threaten the survival of these long-persisting species.

The pattern of deep divergences found between the high elevation Sunda squirrels (Den Tex et al., 2010), is mirrored in the high elevation plain long-nosed squirrels. High elevation Sunda squirrels had an average age of sister-species divergence estimated at 4.04 MYA (*Sundasciurus rabori*, 2.7 MYA, *S. tahan-S. altitudinus*, 3.72 MYA, *S. fraterculus-S. lowii*, 2.29 MYA, *S. brookei*, 6.01 MYA, and *S. jentinki* 5.5 MYA), similar to the 4.4 MYA estimated here for plain long-nosed squirrels. These dates are much older than the average age calculated for low-elevation species (*S. lowii*, 1.28 MYA, *S. tenuis*, 4.73 MYA, *S. hoogstraali*, 0.23 MYA, *S. juvencus*, 0.17 MYA, *S. moellendorffi*, 0.13 MYA, *S. samarensis-S. mindanensis*, 0.94 MYA, *S. philippinensis*, 1.2 MYA, *S. steeri*, 0.61 MYA, and *S. hippurus*, 1.27 MYA), which averaged to 1.2 MYA. This could imply a different rate of speciation and/or extinction for species at low versus high elevations. The Philippines represent a very recent colonization of squirrels, although other rodents show a much deeper history on the Philippines, and have also colonized high elevation habitats multiple times (Justiniano et al., 2015).

The ancestral range reconstruction revealed a complex history of movement from Asia to the Sunda shelf, with subsequent colonizations in nearly all possible directions. We recovered at least four colonizations of Borneo, and likely five colonizations of the Malay Peninsula, three to Sumatra, and two into the Philippines. There was also evidence of movement back into Mainland Asia from the *Callosciurus* species included, which may have a Sundaland origin. This highlights the dynamic history of speciation, analogous to the dynamic history of differentiation between populations within species (Leonard et al., 2015).

Overall, the BMGS represents an endemic lineage adapted to the highlands of Borneo, an area that is home to a large number of montane endemics. The molecular phylogeny has revealed the ancestry of the BMGS is not in the plain long-nosed squirrels, but rather with the Sunda squirrels. The re-classification of the BMGS (*Sundasciurus everetti*) serves as a reminder that phylogenetic relationships, species boundaries, and geographic distributions within most genera of tropical Asian squirrels remain sparsely studied, obscuring the true evolutionary and biogeographic history, taxonomic diversity, and conservation status of this rich mammalian radiation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jmpev.2015.10.023>.

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